

What is claimed is:

1. A serum-free cell-freezing medium consisting essentially of a virus-production serum-free medium (VP-SFM) supplemented with (a) an enzymatic hydrolysate  
5 cryostabilizer selected from the group consisting of soy hydrolysate and rice hydrolysate, added at about 2 g per liter of said medium, and (b) dimethylsulfoxide (DMSO).
2. The serum-free cell-freezing medium of claim 1, which is supplemented with  
10 about 10% DMSO.
3. A process for generating a stable serum-free Vero cell bank, which process comprises the steps of:
  - (a) initiating a culture, which comprises thawing frozen Vero cells and adding  
15 them to growth medium in a T-150 cm<sup>2</sup> flask, wherein the growth medium consists of VP-SFM with 4mM L-glutamine, incubating the cells overnight at about 37°C and 5% CO<sub>2</sub>, and refeeding the culture with fresh growth medium the following day;
  - (b) propagating and amplifying the cells, which comprises growing the cells to confluence in the T-150 cm<sup>2</sup> flask incubated at about 37°C and 5% CO<sub>2</sub>, removing  
20 the medium, washing the flask phosphate buffered saline (PBS) without calcium and magnesium, adding trypsin to the flask and incubating at room temperature for a sufficient time to dislodge the cells from the flask, neutralizing the trypsin with soybean trypsin inhibitor (STI) and adding VP-SFM for nutritional support, seeding the resulting suspension into five T-150 cm<sup>2</sup> flasks and adding VP-SFM to each flask  
25 to a level of 50 ml, incubating the cells at about 37°C and 5% CO<sub>2</sub> for three to four days, pooling the cell suspensions from the five flasks, seeding a cell factory with the pooled suspension, refeeding the cell factory, and harvesting the cell factory; and
  - (c) freezing the cell bank, which comprises centrifuging the harvested cells from the cell factory for 10 minutes at 210xg at 4°C, resuspending the cells in the serum-  
30 free cell-freezing medium of claim 1 at a density of  $2 \times 10^6$  to  $2 \times 10^7$  cells/ml, dispensing the cell suspension into cryovials at one ml of cell suspension per vial, freezing the cells using an active rate control freezer, and storing the cells in liquid nitrogen,

wherein the stable serum-free Vero cell bank thus produced has a cell viability of at least 80% and a recovery doubling time between 40 and 60 hours after one year.

4. A process for generating a stable serum-free Vero cell bank, which process  
5 comprises the steps of:
- (a) initiating a culture, which comprises thawing  $2 \times 10^7$  frozen Vero cells and  
adding them to 50 ml of growth medium in a T-150 cm<sup>2</sup> flask to obtain a cell density  
of  $4\text{--}5 \times 10^5$  cells/ml, wherein the growth medium consists of VP-SFM with 4mM L-  
glutamine, incubating the cells overnight at 37°C and 5% CO<sub>2</sub>, and refeeding the  
10 culture with fresh growth medium the following day;
- (b) propagating and amplifying the cells, which comprises growing the cells to  
confluence in the T-150 cm<sup>2</sup> flask incubated at 37°C and 5% CO<sub>2</sub>, removing the  
medium, washing the flask two times with 20 ml phosphate buffered saline (PBS)  
without calcium and magnesium, adding 5 ml trypsin to the flask and incubating at  
15 room temperature for a sufficient time to dislodge the cells from the flask, neutralizing  
the trypsin with 5 ml of soybean trypsin inhibitor (STI) and adding 10 ml modified VP-  
SFM for nutritional support, seeding the resulting suspension into five T-150 cm<sup>2</sup>  
flasks at a concentration of  $4 \times 10^4$  cells/cm<sup>2</sup> and adding VP-SFM to each flask to a  
level of 50 ml, incubating the cells at 37°C and 5% CO<sub>2</sub> for three to four days, pooling  
20 the cell suspensions from the five flasks, seeding a cell factory with the pooled  
suspension, refeeding the cell factory, and harvesting the cell factory; and
- (c) freezing the cell bank, which comprises centrifuging the harvested cells from  
the cell factory at for 10 minutes at 210xg at 4°C, resuspending the cells in the  
serum-free cell-freezing medium of claim 2 at a density of  $1\text{--}2 \times 10^7$  cells/ml,  
25 dispensing the cell suspension into cryovials at one ml of cell suspension per vial,  
freezing the cells using an active rate control freezer, and storing the cells in liquid  
nitrogen,  
wherein the stable serum-free Vero cell bank thus produced has a cell viability of at  
least 80% and a recovery doubling time between 40 and 60 hours after one year.  
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5. The serum-free cell-freezing medium of claim 1 or 2, wherein the enzymatic  
hydrolysate cryostabilizer is a soy hydrolysate.

6. The serum-free cell-freezing medium of claim 1 or 2, wherein the enzymatic hydrolysate cryostabilizer is a rice hydrolysate.
7. A stable serum-free Vero cell bank having a cell viability of least 80% and a  
5 recovery doubling time between 40 and 60 hours after one year.
8. A stable serum-free Vero cell bank having a cell viability of least 80% and a  
recovery doubling time between 40 and 60 hours after one year, wherein the cell  
bank is produced by the process of claim 3 or 4.

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